Towards a better understanding of human eye disease: insights from the zebrafish, *Danio rerio*

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Abstract

Visual impairment and blindness is widespread across the human population, and the development of therapies for ocular pathologies is of high priority. The zebrafish represents a valuable model organism for studying human ocular disease; it is utilized in eye research to understand underlying developmental processes, to identify potential causative genes for human disorders, and to develop therapies. Zebrafish eyes are similar in morphology, physiology, gene expression and function to human eyes. Furthermore, zebrafish are highly amenable to laboratory research. This review outlines the use of zebrafish as a model for human ocular diseases such as colobomas, glaucoma, cataracts, photoreceptor degeneration, as well as dystrophies of the cornea and retinal pigmented epithelium.

Keywords

zebrafish; eye; ocular; disease; retina; lens; RPE; cornea; glaucoma; vasculature

I. Introduction

A. Overview

Visual impairments affect over 160 million people world-wide, of whom 37 million are blind [1]. Major causes of blindness include cataracts, glaucoma, age-related macular degeneration, and diabetic retinopathy, and the development of effective therapies for these disorders is of high priority. Model organisms with similar physiology to humans are vital to understand underlying developmental processes, identify potential causative genes for human disorders, and develop therapies.

This review focuses on the zebrafish as a model organism for studying human eye diseases. The zebrafish is a remarkably amenable model organism for scientific research, and it combines advantages characteristic of invertebrate models with those inherent to vertebrates for modeling human physiology. As will be explained in the following sections, the zebrafish eye is similar in morphology, physiology, gene expression, and function to the human eye. Zebrafish researchers utilize mutant zebrafish recovered from forward genetic screens along with a number of reverse genetic techniques to model many of the major eye diseases which plague humans (Table 1).

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B. The Zebrafish as a Model Organism

The zebrafish, *Danio rerio*, is a common aquarium fish that originated in the Ganges region of India [2]. The rapid increase in its popularity for the study of vertebrate development and genetics is mainly due to its transparent embryos that develop *ex-utero*, making the visualization of developmental events possible. In addition, its large clutch sizes and ease of maintenance, features that are commonly associated with invertebrate model organisms such as *Drosophila*, make it an attractive alternative to model species such as the mouse. Zebrafish have a short generation time of 2–4 months and a single mating pair can produce around 200 offspring on a weekly basis [3]. These, combined with the low relative cost of raising zebrafish, make it an ideal model organism as evidenced by the rapid growth of the zebrafish research community.

Since its introduction as a model organism three decades ago, advances in available technologies pertaining to zebrafish research have quickly closed the gap with those available for other model organisms (see 'Tools and Techniques' section). Importantly, the Zebrafish Information Network (ZFIN) maintains a central comprehensive online database that provides researchers with access to information and services that include gene expression pattern databases, mutant and transgenic lines, and links to a fully sequenced genome (www.zfin.org).

C. Tools and Techniques

External development of zebrafish embryos provides the opportunity for various manipulations from the time of fertilization. These include the injection of tracer dyes or of mRNA (for transient expression of protein). Established fate-maps [4, 5] enable the transplantation of labeled blastula- or gastrula-stage donor cells into specific regions of host embryos. When the donor and host are of different genetic backgrounds, the resulting chimeric zebrafish can be used to determine whether a gene whose mutation is embryonic-lethal early in development has a role later in the life of the organism, or to establish the cell- or tissue-autonomy of a mutant phenotype [6]. Of particular relevance to this review, entire lenses can be transplanted between embryos to determine lens- versus retinal-contribution to an ocular phenotype [7].

1. Targeted Knockdown of Gene Expression—Homologous recombination strategies utilized in other model organisms to introduce specific mutations into the genome are not yet available in zebrafish. However, specific and heritable targeted mutagenesis has recently been effected with zinc-finger nucleases [8, 9]. Additionally, transient gene knock-down is commonly performed in zebrafish by injection of morpholinos to block gene expression [10, 11]. Morpholinos are effective and inexpensive oligomeric constructs designed to bind mRNA and prevent proper splicing or translation. Morpholinos are effective for several days, and their transient activity is sufficient for the embryonic timeframe of many zebrafish experiments. Morpholinos can also be introduced into specific tissues later in development by electroporation [12], and photoactivatable morpholinos (which can be activated in specific cells of transparent embryos by a laser) allow precise spatiotemporal control of gene knock-down [13]. Thus, zinc finger nucleases and morpholinos complement the catalog of mutant fish lines generated from mutagenesis screens.

2. Transgenic Techniques—Transient or stable introduction of transgenes in zebrafish is effected in a number of ways. Injection of DNA into a fertilized egg results in mosaic, transient expression in the embryo and low-efficiency integration into the genome [14–16]. Tol2 transposon-mediated strategies result in much higher efficiency transgene integration [17], and transgenic lines of zebrafish are routinely created by utilizing this technique. Specific spatiotemporal control of transgene expression is achieved by placing the transgene

under the control of a ubiquitous promoter [18], heat-shock promoter [19], or tissue-specific enhancer/promoter elements [20]. In addition to spatiotemporal control over expression of genes of interest, these techniques may be utilized to express dominant negatives [21] or genetically-encoded reporter constructs such as GFP [18]. Genetically-encoded photoconvertible proteins, such as Kaede, may be utilized for lineage-tracing experiments [22]. Transgenes can also be used to ablate specific cells by expression of either diptheria toxin [23] or nitroreductase (in which case cell-autonomous ablation is not elicited until the application of a prodrug) [24].

Many of the transgenic tools originally implemented in mouse and Drosophila research are also utilized in zebrafish. The Gal4-UAS system for ectopic gene expression involves spatiotemporally-controlled transcription of *gal4* under a promoter of choice. The Gal4 protein then binds to and activates expression of genes downstream of its upstream activation sequence (UAS) [20, 25, 26]. Cre-LoxP recombination [27], which allows for excision or inversion of a segment of transgene DNA upon activation of Cre, has also been utilized in zebrafish [28]. Cre-recombinase can be activated in zebrafish in many ways, including most recently the photo-uncaging of 4-OH-cyclofen for activation of a ligand-inducible Cre [29]. Therefore, the future of zebrafish transgenesis is extremely exciting.

D. Genetic Screens

Large-scale genetic screens in *Drosophila* and *C. elegans* have identified numerous genes required for embryonic development [30, 31]. Similar approaches were thought to not be feasible in vertebrates due to long generation times and small number of progeny of traditional vertebrate models such as the mouse and chick [3]. However, the pioneering work of George Steisinger nearly three decades ago established the zebrafish as a powerful genetic model organism for the identification of genes important for vertebrate development [32, 33]. Two large-scale genetic screens performed in Christiane Nusslein-Volhard and Wolfgang Driever's labs followed fifteen years later and were published in a special issue of the journal *Development*, describing mutations affecting various aspects of vertebrate development [34, 35]. Since then, multiple large- and small-scale mutagenesis efforts have produced numerous mutant lines that have been studied to better understand vertebrate development, as well as disease.

Most genetic screens in zebrafish utilize the chemical mutagen ethylnitrosourea (ENU) due to its high mutagenic efficiency (Figure 1A) [36, 37]. Like ethyl methanesulphonate (EMS), which is commonly used in genetic screens in *Drosophila*, ENU is an alkylating agent that produces point mutations in the adult germline. While ENU mutagenesis has proved to be a powerful method for the induction of point mutations in the zebrafish, the identification of the mutated gene can be laborious. Positional cloning of a single mutation requires the work of one researcher for approximately six to twelve months [38]. A separate screen performed in Nancy Hopkins' lab used retroviral insertional mutagenesis as an alternative (Figure 1B). Since the genetic sequence of the insertion is known, the location of the retroviral insertion can be assayed using standard PCR techniques, quickly and reliably revealing the affected gene [39, 40]. While the efficiency of mutagenesis using retroviral insertion is estimated to be about one-seventh that of ENU mutagenesis, the cloning of one mutation requires only three to four weeks to perform [38]. The main disadvantage of insertional mutagenesis is that, due to low efficiency, larger screens need to be performed as compared to ENU-based screens in order to identify equal numbers of mutants. As a result, the need for larger facilities make these screens impractical for most academic labs.

The identification of ENU-induced point mutations can be simplified using a technique known as TILLING (Targeted Induced Local Lesions in Genomes). Unlike traditional screens in which mutants are isolated in the F3 generation and then positionaly cloned, with

TILLING mutations can be identified in the F1 generation. Mutagenized males are bred to generate large populations of F1 offspring whose DNA is analyzed by PCR in order to identify mutations in a gene of interest. A mutation of interest is then isolated by out-crossing a single identified carrier [41]. This approach has generated over 150 loss-of-function alleles yet to be published [42]. In addition, a consortium has been recently established to consolidate TILLING efforts, greatly improving the likelihood of finding mutations of interest in the zebrafish genome. Researchers can now place requests for mutations in specific genes online (www.sanger.ac.uk/projects/D_rerio/mutres).

Mutants isolated from genetic screens can further be used to screen for small molecules that might suppress the mutant phenotype. These compounds can then be further studied as potential therapeutic agents for a particular defect or disease [43]. Small molecule screens, like genetic screens, utilize the already mentioned advantages of zebrafish: large clutch size, rapid development, and transparency of the embryos allowing for the rapid screening of compounds that affect certain aspects of development. The dosage, as well as the time during development of administration can be controlled and the effects on embryonic development assayed by various methods such as *in-situ* hybridization, vital dyes and transgenics to visualize effects on specific tissues, as well as behavioral assays [44–47]. Since the first large-scale small molecule screen was published ten years ago [48], multiple screening efforts have identified compounds that affect various biological processes including cell cycle and cancer, control of stem cell populations, and the formation of retinal vasculature [45, 46, 49].

E. Eye Development and Anatomy

The zebrafish has long been recognized as a useful model for the study of human ocular development and disease [50–53]. Detailed characterization of the embryonic development of the posterior segment of the eye, which includes the neural retina [54] and the RPE [55] and the anterior segment (which includes the lens, cornea, ciliary body, and the various tissues of the iridocorneal angle [56–68]), has not only shed light on the sequence of events in vertebrate eye development, but has also highlighted the similarities in the architecture of the zebrafish eye to that of the human eye.

In zebrafish, eye development is rapid. The optic vesicle, which will ultimately give rise to the neural retina and the retinal pigment epithelium, evaginates from the forebrain at around 12 hours post fertilization (hpf) and remains attached to and continuous with the forebrain through a transient structure called the optic stalk (Figure 2). The optic vesicle then gives rise to the optic cup through a series of morphogenetic events that occur from about 16 hpf to 20 hpf [68]. Morphogenesis of the optic cup continues as the optic fissure forms ventrally by 24 hpf and subsequently closes by 48 hpf. Neurogenesis begins at 28 hpf and by as early as 72 hpf zebrafish embryos exhibit visual function [67].

The anterior segment of the embryonic eye develops concurrently with the events mentioned thus far. At 16 hpf, surface ectoderm cells overlying the optic cup thicken to form the lens placode ([57], Figure 3), the lens mass delaminates from the surface ectoderm at approximately 24 hpf, and fully detaches by 26 hpf [57, 59, 60, 67, 68]. The surface ectoderm overlying the lens becomes the corneal epithelium, which is two cell layers thick by 30 hpf [62]. Migratory periocular mesenchymal cells (which first enter the enter the anterior chamber of the eye at 24 hpf) coalesce to form the corneal endothelium between 30 and 36 hpf [60, 62, 67, 68].

Humans are a diurnal species, and day-time vision is predominantly mediated by cone photoreceptors in the retina. In contrast to nocturnal mice and rats, whose retinas contain few cones, larval zebrafish vision is mediated almost entirely by cone photoreceptors [69].

As in humans, the mature zebrafish retina is composed of three nuclear layers separated by two plexiform layers. Zebrafish possess four types of cones (blue, UV, and red/green double cones) and one rod cell type [70]. Rod and cone cell bodies reside in the outer nuclear layer (ONL), while the inner nuclear layer (INL) is occupied by amacrine, horizontal, bipolar cells, and Müller glia. Visual signals originating in the photoreceptors are transmitted through the retina to the ganglion cells, which make up the ganglion cell layer (GCL); their axons then relay the signal to the brain [54, 71].

II. Posterior Segment

A. Coloboma

Early during vertebrate eye development, the optic vesicle evaginates from the forebrain and ultimately forms the optic cup (see introduction). As a result of a series of morphogenetic processes that follow, a transient gap called the optic fissure forms ventrally and subsequently closes by the fusion of the surrounding tissue [68, 72]. Failure of optic fissure closure is the underlying cause of colobomas, which ultimately affect one or more parts of the eye including retina, choroid, iris cornea, lens, ciliary body, and optic nerve [73]. In humans, the incidence of coloboma is estimated at around 1 per 10,000 births [74–76]; severe colobomas may cause as much as 10% of childhood blindness [77]. The clinical presentations of colobomas are varied, as numerous genetic loci have been associated with this diverse class of ocular pathologies [78]. While the causes underlying most human coloboma conditions remain unknown, studies in zebrafish have contributed to our understanding of the developmental bases of optic fissure closure.

A gene expession profiling study was conducted recently by Brown et al to identify genes whose transcript levels are enriched in the optic fissure of the mouse. Two related genes, *Nlz1* and *Nlz2*, were shown to be expressed in the optic fissure; subsequent morpholino knockdown of both in zebrafish resulted in coloboma [79]. Brown, et al demonstrated that *nlz1* and *nlz2* may function in the optic fissure by directly regulating the expression of the transcription factor *pax2a*. In *nlz1/2*-deficient embryos, *pax2a* expression was drastically reduced, and both the nlz1 and nlz2 proteins bound the *pax2* promoter *in vitro*, suggesting direct transcriptional regulation. In humans, mutation in the *PAX2* gene result in coloboma as part of renal-coloboma syndrome (OMIM 120330) [80], and the human *PAX2* and zebrafish *pax2a* genes are expressed in the developing optic stalk and optic fissure [81, 82]. Interestingly, the zebrafish *No-isthmus (noi)* mutant line, which carries a non-sense mutation in the *pax2a* gene, exhibit optic fissure closure defects, resulting in coloboma [81].

Additional studies in zebrafish have shown that *pax2a* is transcriptionally regulated *by Sonic hedgehog (Shh)*, a well-studied developmental morphogen. In the zebrafish mutant *cyclops*, in which a mutation affecting the nodal pathway results in a deficiency in *Shh* signaling, *pax2a* transcript levels are highly reduced in the optic stalk [83]. In *blowout (blw)* mutants, which possess a loss-of-function mutation in the *patched1* gene, the expression domain of *pax2a* is increased, resulting in an enlarged optic stalk and colobomas [84, 85]. The Patched1 protein is the receptor and negative regulator of the Hedgehog pathway; mutations in patched genes are known to result in an overactive Hedgehog pathway [86]. Interestingly, colobomas have been described in a family carrying specific deletions in the human SHH gene [87], suggesting that the genetic interaction between *Shh* and *pax2a* in zebrafish might be conserved in humans. This interaction was recently shown in zebrafish to be mediated by *zic2a*, a transcription factor whose activity is controlled by *Shh*. *zic2a* restricts *pax2a* expression to the optic stalk, and loss of *zic2a* activity results in the expansion of *pax2a* expression into the retina, resulting in coloboma [88]. While human *ZIC2* mutations have not yet been associated with the formation of coloboma, they have been linked to holoprosencephaly, a forebrain defect frequently associated with colobomas [88, 89].

Studies in zebrafish have therefore identified *ZIC2* as an additional candidate gene in human colobomas.

The Hedgehog pathway has also been shown to control the expression of *vax* genes (*vax1* and *vax2*), whose function has also been shown to be important for the proper development and specification of the optic stalk and ventral retina, tissues in which they are normally expressed [90, 91]. Morpholino knockdown of both *vax1* and *vax2* in zebrafish resulted in the loss of optic stalk and ventral retinal tissue identity, and the presence of colobomas. Over-expression of Shh results in the expansion of *vax* gene expression domains, while blocking the Hh pathway reduces *vax* gene expression. While Shh appears to control the expression of both *pax2a* and *vax* genes, these likely act in parallel to specify optic stalk identity, as the experimental loss of *pax2*a does not lead to transcriptional loss of *vax* genes, and vice versa [91]. Since both *pax2a* and *vax* genes encode transcription factors, it remains to be seen what factors might act downstream of *pax2a* and to directly control the proper development of the optic stalk and ventral retina.

While the Hedgehog pathway seems to play a central role in dictating optic stalk identity and proper optic fissure closure, other developmental pathways have been implicated in proper morphogenesis of the eye. The requirement of retinoic acid (RA) during ocular development, for example, is well established. RA is derived from vitamin A, which has been linked to ocular pathologies in humans, including coloboma [92]. In zebrafish, exposure to exogenous RA early during eye development results in an enlarged optic stalk and, depending on the dose of RA, duplication of the ventral retina [93, 94]. Conversely, embryonic exposure to citral, which blocks RA synthesis, results in optic stalk and ventral retinal loss and ocular coloboma [95]. In addition, morpholino knockdown of *bcox*, an enzyme critical for the synthesis of RA, results in numerous developmental phenotypes, including coloboma [96].

A possible relationship between RA synthesis and ocular colobomas has emerged through the characterization of the zebrafish *adenomatous polyposis coli* (*apc)* mutant. Human mutations in *APC*, a well studied tumor suppressor, underlie familial adenomatous polyposis syndrome [97]. These patients suffer from failure in the development of the ventral retina and coloboma, phenotypes that are also found in zebrafish *apc* mutants. Through elegant gain- and loss-of-function experiments, Nadauld et al demonstrated that Apc regulates RA synthesis indirectly through the regulation of Rdh5, an enzyme responsible for RA synthesis in the ventral retina. These findings suggest that a deficiency of retinoic acid, a vitamin A derivative, might underly ocular phenotypes in familial adenomatous polyposis syndrome [98].

The expression of RA synthesizing enzymes in the optic vesicle and optic cup in zebrafish, chick, and mouse suggests that RA is required autonomously in the eye for proper ocular development [99–101]. However, surprisingly, studies in mouse have demonstrated that RA activity in the neural crest-derived periocular mesenchyme (POM) is responsible for early eye morphogenesis [102]. Consistent with these findings, migration defects and subsequent apoptosis of POM in the zebrafish *lmx1b* morphant lead to morphogenetic defects in the optic fissure and colobomas. Loss of *lmx1b* results in a transient up-regulation of Fgf signaling, which in turn at least partially affects proper patterning and morphogenesis of the embryonic eye [103].

The developmental pathways discusses thus far most likely regulate optic fissure closure indirectly, through the regulation of other factors. Studies in zebrafish have also suggested that the molecular mechanisms underlying optic fissure closure are likely to involve interactions between the cells of the retina/optic stalk with the extracellular matrix (ECM).

Mutations in *laminin β1* and *γ1* (*lamβ1* and *lamγ1)*, for example, result in disruptions in basement membrane integrity and the presence of a number of ocular phenotypes including coloboma [104, 105]. Laminins are major components of the ECM, which serves as a scaffold for tissue morphogenesis, as well as a site for the deposition of growth factors such as Fgf and Hedgehog ligands. In the future, it would be interesting to determine whether ocular phenotypes in laminin mutants might be a result of misregulation of these signaling pathways and/or a consequence of morphogenetic defects due to perturbed cell-ECM interactions.

B. Photoreceptors

Photoreceptor degenerations are the most common form of blindness in the Western world and involve the loss of vision due to dystrophy and/or death of retinal photoreceptors. These pathologies can be roughly divided into those conditions that initially affect rod photoreceptors, such as retinitis pigmentosa (RP), and those that initially affect cone photoreceptors, such as macular degeneration [53]. While photoreceptor degeneration can also be caused indirectly by primary defects in the RPE, which serves an important function in photoreceptor health and homeostasis, this topic will be discussed in a later section (see 'RPE').

Photoreceptors are highly polarized sensory cells that consist of an inner segment (IS) that is connected to an outer segment (OS) by a highly modified cilium (Figure 5) [106]. In mature photoreceptors, proteins that are required for growth and maintenance of photoreceptor OS's, as well as for phototransduction, are transported along polarized microtubules from the basal IS to the apical OS. Photoreceptors are similar to epithelial cells in that their surfaces are divided into apical and basolateral domains by cell junctions. Their centrosomes are located at the apical surface, while their nuclei reside basally [107]. Proper development and subsequent maintenance of vertebrate photoreceptors relies on the establishment of proper apical-basal polarity, as well as the function of the transport machinery. It is therefore not surprising that of the more than 100 genetic loci known to cause photoreceptor degenerations in vertebrates, most affect the structure and function of the OS [108].

Genetic screens in zebrafish have shed light on the molecular bases of these cellular functions by the isolation of mutants in which photoreceptor development and/or maintenance are defective [104, 109, 110]. One such mutant, *mosaic eyes (moe)*, was found to contain expanded apical features in photoreceptors [111, 112]. The *moe* locus encodes a FERM-domain containing protein that forms a complex with Crumbs proteins, which in turn have been shown to be critical for apical-basal polarity in *Drosophila,* as well as vertebrates [113–115]. Interestingly, mutations in the human orthologue of Crumbs, *CRB1*, are associated with retinal photoreceptor dystrophies such as retinitis pigmentosa 12 [116] and Leber's congenital anamurosis (LCA1, [117, 118]). Other zebrafish mutants, such as *nagie oko (nok)* and *heart and soul (has)* do not only phenocopy the *moe* mutant phenotype, but the proteins that they encode (Pals1 and PKCi, respectively) also interact biochemically with Moe [111]. Moreover, morpholino knockdown of *crb2b*, a zebrafish Crumbs paralog, results in the reduction of IS size [119]. These studies have therefore begun to uncover the role of the Crumbs complex in vertebrate photoreceptor development and disease.

The formation of correct apical-basal polarity depends not only on the proper function of cell polarity determinants, but also on their transport and localization. Photoreceptors in the *ale oko (ako)* mutant retina accumulate the apical determinants Pals1 and PKCλ in their cell bodies and exhibit extensive photoreceptor death late in development [120]. The *ako* locus encodes the p50 subunit of the dynactin complex, which serves an important role in the transport of cargo along microtubules as part of the dynein motor complex [121]. In another mutant, *mikre oko (mok)*, which possesses a mutation in *dynactin-1*, retinal degeneration is

at least partially due to mislocalization of photoreceptor nuclei. Surprisingly, unlike *ako* mutants, apical determinants such as Crumbs and aPKCλ are not mislocalized, suggesting that degeneration in these mutants is not due to loss of cell polarity. Experimental mislocalization of the nucleus by the overexpression of the dynein motor resulted in photoreceptor cell death, supporting the role of nuclear position in photoreceptor survival [122]. While nuclear positioning defects in *mok* photoreceptors seems to have a cell-autonomous role, other non-cell autonomous components seem to play a role in *mok* degeneration. Mosaic analyses have revealed that mutant photoreceptors display over a 2.5 fold increase in survival when placed in a wild-type environment [123]. This finding suggests that photoreceptor health and survival depends on environmental cues from surrounding cells. These findings are consistent with defects associated with some human degenerations, such as retinitis pigmentosa, where an initial defect in the rod specific rhodopsin ultimately results in the loss of both rods and cones [124].

Transport from the cell body to the OS is also important to replace OS components that are lost due to the continual phagocytosis of photoreceptor OS's by the overlying RPE [125]. In photoreceptors, as in cilia, transport occurs by a process known as Intraflagellar Transport (IFT) and mutations in IFT component genes such *ift57, ift80, ift88, and ift172* result in OS atrophy and/or complete loss of OS's in zebrafish [104, 126, 127]. Mutations in the *elipsa* gene, which encodes a protein that is thought to facilitate IFT [128], result in early photoreceptor loss [129], while morpholino knockdown of multiple subunits of the retrograde IFT motor dynein-2 has highlighted the importance of IFT in proper OS elongation and visual function [130]. Photoreceptor degenerations relating to defective IFT are some of the many cilia related phenotypes associated with Bardet-Biedl Syndrome (BBS) [131], and the further study of the function of IFT components in zebrafish might prove to be instrumental in gaining a better understanding of the molecular causes underlying BBS-related ocular pathologies.

While the mutants described thus far in this section were isolated in genetic screens utilizing mainly histological methods to detect photoreceptor defects, others were isolated in behavioral screens [132–135]. One such screen took advantage of the ability of zebrafish to exhibit vision-dependent behavior as early as 3dpf. Brockerhoff et al first used the optokinetic response assay (OKR) on mutagenized embryos to detect defects in visual function. A second assay involved the use of electroretinogram (ERG) recordings to identify whether isolated mutations affect outer retinal function [133]. From this screen, 18 mutants were isolated that were determined to have reduced visual function. One, *no optokinetic response a (noa),* possessed no gross photoreceptor abnormalities at 5dpf, but was found to be blind, as well as lethargic, and died prematurely. Analysis of the *noa* mutation revealed a deficiency in a subunit of the pyruvate dehydrogenase (PDH) complex, which regulates energy production in cells. The *noa* mutant line has been used as a model for the study of PDH deficiency (OMIM 245348), a human disorder that, like the zebrafish model, results in blindness, neurological defects, and early death [136]. Since current treatments for PDH deficiency in humans have resulted in limited success, Taylor et al utilized *noa* mutants to test a ketogenic diet which has shown some success in alleviating PDH deficiency symptoms in a limited number of human patients. Administration of this special diet restored normal behavior in *noa* mutants, highlighting the potential of studying PDH deficiency therapies in the *noa* mutant line.

Another mutant, *no optokinetic response f (nof)* was found to possess a mutation in the α subunit of cone transducin (Tcα), a G-protein required for phototransduction. In human patients suffering from a condition known as achromatopsia (OMIM 139340), mutations in Tcα underlie loss of color vision. In *nof* mutants, cone development occurs normally; they are, however, up to 1000x less sensitive to light, as detected by recordings of single

photoreceptors. Extensive analysis of cone light responses revealed that residual phototransduction is light-dependent, but transducin independent. Ca^{+2} influx, which is important for photoreceptor light adaptation and was previously thought to be controlled by transducin, was still detectable in *nof* mutants. This study therefore revealed that some Ca^{+2} influx in cone photoreceptors might be transducin independent.

In a separate screen, dominant mutations that cause photoreceptor degeneration in adult zebrafish was performed in order to isolate genetic mutants that could be later used to study human inherited night blindness such as retinitis pigmentosa (RP) [137, 138]. In the case of RP, many genetic loci underlie this group of disorders. However, only about half of the cases of dominant RP have been linked to specific mutations at the time the screen was performed [138]. Li et al therefore screened mutagenized adult zebrafish by utilizing a known escape response exhibited by the fish. A lack of such response to a threatening cue was interpreted as a loss of vision, which was later confirmed using ERG recordings. In all, seven heterozygous mutants were isolated (*nightblindness a,b,c,d,e, and f*), out of which six exhibited photoreceptor degeneration [138–141]. Four of the six were found to be embryonic lethal as homozygotes, suggesting that the mutated genes underlying the photoreceptor phenotypes have other critical functions during embryonic development. This finding highlights the importance of such screens for the identification of dominant mutations that might not be easily isolated in traditional screening due to early embryonic lethality.

C. RPE

The RPE is a monolayer of pigmented cells that serves in the protection and maintenance of photoreceptors, and is therefore essential for visual function. In addition to its role in absorbing excess light entering the eye, the RPE transports essential nutrients from the blood to photoreceptors, while removing ions, water, and metabolic end products from the retina to the blood. Vertebrate phototransduction depends on the conversion of all-*trans*-retinal to 11-*cis*-retinal through a series of biochemical reactions termed the retinoid cycle, and most of these essential reactions are carried out by the RPE rather than photoreceptors [142]. Finally, the RPE supports photoreceptor renewal by the daily phagocytosis of about 10% of OS volume, while the photoreceptor itself regenerates roughly the same volume each day to maintain proper OS length [143]. Failure of any of these functions often results in retinal degeneration and loss of vision ([144]).

An important question in the study of many types of retinal degenerations is whether photoreceptor degeneration occurs due to a primary defect in photoreceptors themselves or as a secondary consequence of RPE degeneration. Since in many cases defects in both photoreceptor and RPE are present, a cause-and-effect relationship cannot be easily established between these cell types. Krock et al studied this relationship in the zebrafish *rep1/chm* mutant line, which exhibits retinal phenotypes consistent with those found in humans suffering from choroideremia, a form of hereditary retinal degeneration associated with mutations in the human REP1 gene [145]. Rep proteins are involved in the posttranslational modification of Rab protein, and are therefore critical for vesicle trafficking. Since both photoreceptors and the RPE depend on vesicle trafficking for transport of material (such as opsin) to the OS and degradation of phagocytosed OS material, respectively, the initial defect due to the *rep1* mutation could potentially lay in either tissue. Through the use of mosaic analysis, Krock et al showed a loss of rep1 in the RPE is sufficient to cause photoreceptor degeneration and results in the localized accumulation of OS material. These findings, together with the observation that opsin was not mislocalized in *rep1* photoreceptors suggest that defective cellular processes within the RPE are the primary cause of photoreceptor degeneration in choroideremia patients. In a separate study however, Moosajee et al showed that zebrafish do not possess an orthologue

of a second human REP gene, *REP2*. *REP2* plays an essential role in the pathogenesis of choroideremia as its function is thought to compensate for the loss of *REP1*. The authors of the study therefore concluded that the *rep1* mutant line may not be a suitable model for the study of choroideremia in its present form [146].

Defects in vesicle trafficking, formation, and/or fusion are known to be the underlying cause of many human ocular diseases such Chediak-Higashi syndrome (CHS, OMIM 214500), Hermansky-Pudlak syndrome (HPS, OMIM 203300), and Griscelli syndromes (GS1-3, OMIM 214450, 607624, and 609227). The pathologies of these human syndromes include hypo-pigmentation of the RPE, as well as loss of visual function. The RPE contains lysosome-related organelles known as melanosomes that synthesize and store melanin, the main pigment present in the melanosomes of the RPE [147]. Proper fusion of lysosome-related organelles depends on the homotypic fusion and vacuolar protein sorting (HOPS) complex, as it is required for SNARE complex assembly. A model for the study of human conditions such as CHS and HPS has emerged recently in the zebrafish *leberknödel (lbk)* mutant line. *lbk* mutants possess a mutation in the *vam6/vps39* gene, which encodes a component of the HOPS complex. Hypopigmentation of the *lbk* RPE, a common characteristic of vesicle traffic defects, results from a defect in melanosome maturation, and the RPE contains vesicles containing undigested photoreceptor OS's. As a result, OS's are shortened and *lbk* mutants display reduced visual function [148]. A mutant in another HOPS complex component, *vps18*, also possesses immature melanocytes and hypopigmentation, and can serve as an additional model for the study of the underlying cellular and molecular basis of ocular defects in hypopigmentation-related disorders [149].

In addition to their role in melanin synthesis, melanosomes are also thought to play a role in the degradation and detoxification of phagocytosed photoreceptor outer segments by the RPE [150]. In human patients suffering from age-related macular degeneration (ARMD), phagocytosed OS's are not properly digested by the RPE. This defect results in the accumulation of the lipofuscin component A2E, which is thought to further inhibit OS component degradation, contributing to the progression of ARMD [151]. It is thought that A2E might inhibit the activity of vacuolar ATPases (v-ATPase), whose known role is the acidification of lysosomes and lysosome-related organelles, such as melanosomes [152]. Acidification of these organelles is, in turn, important for the activity of proteases and therefore for proper degradation of accumulated materials. Consistent with this model, a recent analysis of five separate zebrafish mutant lines defective in different subunits of the v-ATPase complex revealed accumulations of undigested OS material in the RPE [153]. While no mutations in v-ATPase have been linked to blindness in humans, these results suggest that defects in v-ATPase might directly or indirectly cause disorders that affect RPE function.

Some of the other mutants that have been shown to possess RPE defects coupled to retinal degeneration are *gantenbein (gnn), bleached (blc), fading vision (fdv)*, and *fade out (fad)* [154–156]. Of those, only the mutation underlying the *fdv* phenotype has been cloned and identified. The *fdv* mutant was originally isolated during a mutagenesis screen due to reduced pigmentation in the eye as well as the rest its body [157]. Like the *lbk, vps18*, and v-ATPase mutants, the *fdv* RPE contains vesicular inclusions and their OS's are reduced. However, the visual defects identified in *fdv* mutants are thought to be due to disrupted recycling of visual pigment, a defect that can be attributed to primary defects in melanosome biogenesis [158]. Positional cloning identified a mutation in the *silver a/pmel17* gene, whose orthologue has been originally shown to be important for pigmentation in mice [159]. While *silver* orthologues in various species have been described and its overall function in melanosome biogenesis established, the molecular mechanisms underlying its roles in pigmentation in general and RPE function specifically are still controversial [160]. Further

study of existing mutants, such as *fdv*, as well as the identification and cloning of additional pigmentation mutants, are therefore critical for our understanding of role of the RPE in ocular development and disease.

D. Müller Glia in Regeneration and Disease

The study of stem cells has been of major interest due to the growing potential of their use in therapeutic and regenerative medicine. The ability of zebrafish to regenerate various tissues after injury has attracted major attention from researchers seeking to identify the cellular and molecular mechanisms that enable this regenerative capacity, which is often absent in mammals [161–163]. In the vertebrate retina, two populations of stem/progenitor cells have been identified: the Müller glia of the central retina, and the ciliary marginal zone (CMZ) at the most peripheral edge of the retina. Both of these stem cell populations add new neurons and glia to the zebrafish retina throughout the lifetime of the animal. Müller glia give rise to rod photoreceptor precursors that migrate along Müller glial processes to the photoreceptor layer. All other retinal cell types are continually produced in the post-embryonic CMZ. While the study of the stem/progenitor populations of the CMZ in zebrafish has seen much progress in the past few years, including the identification of mutants with CMZ-specific phenotypes [104, 164–168], the remainder of this section will focus on recent findings regarding Müller glia.

Neuroprotection, maintenance of retinal homeostasis, and the establishment of proper retinal lamination are some of the many functions attributed to Müller glia in the healthy retina ([169]). In response to retinal disease or damage, Müller glia can become 'reactive', as characterized by changes in gene expression that are often followed by de-differentiation and proliferation [170, 171]. Virtually every human retinal disease is associated to some degree with Müller glial reactivity [169]. In some cases, such as diabetic and proliferative retinopathies, and retinal detachment, Müller glia are thought to become reactive in response to primary detects arising in another cell type. In others, Müller glia are thought to be the primary cell type affected. Still, in many human eye diseases, the role of Müller glia is the onset of the disease is unclear. In humans suffering from Basal Cell Naevus Syndrome (BCNS), epiretinal membranes (ERMs) - proliferative and structural abnormalites at the boundary between the retina and the vitreous, have been shown to contain a major glial component. Studies in *Ptch* mutant mice have shown that Müller glial reactivity is locally associated with BCNS-related ocular pathologies [172]. However, it is yet unclear whether ocular defects in BCNS patients are a result of a primary defects at the level of Müller glia, or conversely, whether Müller glia are reactive in response to another ocular defect. Recently, a zebrafish model for the study of BCNS-related ocular pathologies has emerged in the *leprechaun (lep)* mutant line, which possesses a loss-of-function mutation in the *patched2* gene, the zebrafish orthologue of *PTCH*. *lep* mutants display retinal abnormalities that are similar to those observed in human BCNS patients, including disruptions at the vitreo-retinal interface [165]. Further study of *ptc2* mutants is required to determine the role of Müller glia in BCNS-related ocular pathologies.

Müller glia have long been known to possess neurogenic potential, having an intrinsic ability to give rise to newborn neurons. In the adult retina of lower vertebrates, differentiated Müller glia continue to express molecular markers that are characteristic of early retinal progenitors, such as *pax6* and *rx1* [173]. Clusters of proliferating rod progenitors are closely associated with Müller glia and appear to migrate along their processes, which span the apical-basal width of the adult retina, to their final location in the photoreceptor layer [174, 175]. A recent study utilizing transgenic zebrafish that express GFP under the control of a Müller glia-specific promoter (Tg(*gfap*:GFP)) as a lineage tracer has shown that rod progenitors retain low levels of GFP, suggesting that Müller glia indeed give rise to rod photoreceptor precursors in the adult retina [176]. These findings support the neurogenic

potential of Müller glia and raise the intriguing possibility of using Müller glia as a source for cell replacement therapies for humans suffering from age-related photoreceptor loss.

While in the uninjured adult retina Müller glia give rise strictly to progenitors destined to the rod lineage, Müller glia are also able to dedifferentiate, proliferate, and replenish all neuronal cell types in response to retinal damage [171, 176]. While conclusive lineage tracing to show Müller glia as the sole source of regenerated neurons have not yet been published, the use of zebrafish transgenic lines together with extensive immuno-histochemical analysis has strongly supported the role of Müller glia as the stem cell population responsible for the regenerative response in the central retina [176, 177]. Since mutant analysis in adults is labor intensive and time consuming, multiple labs have also performed microarray analyses after retinal damage in order identify genes that play a role in these processes. Consistent with the idea that regenerative processes often re-capitulate embryonic development, these studies identified known developmental pathways such as BMP, Notch, Wnt and Hedgehog as being transcriptionally regulated in reactive Müller glia [178, 179]. It remains to be seen what exact roles of each of these pathways in retinal regeneration, and whether the genetic and/or chemical manipulation of these pathways might have therapeutic value in humans.

E. Intraocular Vasculature

The vertebrate eye is highly metabolically active, and the intraocular vasculature provides oxygen and other nutrients [180, 181]. Many human diseases present with defects in intraocular vascularization, including diabetic retinopathy, retinopathy of prematurity, and age-related macular degeneration [181]. Similarities between zebrafish and human intraocular vasculature make the zebrafish an exciting new model organism in this field. For instance, Alvarez and colleagues [182] recently showed that a zebrafish model of hyperglycemia [183] recapitulates some aspects of non-proliferative diabetic retinopathy; zebrafish may therefore be a useful model for this very common human disease.

In humans and in zebrafish, the hyaloid vessel network surrounds the posterior lens during development [184, 185]. In humans, the hyaloid vasculature regresses completely by birth, during which time the retinal vasculature (which lines the inner limiting membrane) forms [184]. In contrast, hyaloid regression does not take place in zebrafish; the hyaloid vasculature instead remodels into the retinal vasculature [185]. In humans and in zebrafish, the choroid vasculature covers the outer surface of the retina [46, 181].

One study utilized a transgenic zebrafish line in which cells of the vasculature express GFP ($Tg(fli1a:EGFP)^{y1}$) to screen various mutant and morphant zebrafish for altered formation of the intraocular vasculature [185]. Utilizing this strategy, the authors demonstrated that several genes are required for hyaloid and retinal vasculature development and maintenance, including *syndecan 2* (morphants had no vasculature on the lens), and *mab21l2* (morphants displayed disrupted hyaloid vessel patterning). Another study which investigated the effects of *foxc1* depletion (by morpholino) on hyaloid vessel ultrastructure found that the integrity of the basement membrane of hyaloid vascular cells was disrupted [186].

Also utilizing transgenic zebrafish lines which express GFP in the vasculature ($Tg(fli1a:EGFP)^{y1}$ and $Tg(kdrl:GFP)^{la116}$), Kitambi and colleagues [46] performed a small molecule screen to search for compounds which altered retinal vasculature without significant alteration in trunk vasculature. The authors reported two classes of compounds: one which induced degeneration of vessels, and one which increased vessel diameter [46]. This study provides an example of the powerful role that zebrafish small molecule screens can play in therapeutics research.

III. Anterior Segment

A. Lens

The lens is a specialized transparent tissue which, in conjunction with the cornea, focuses incoming light onto the retina [187]. Lens opacity, or cataract, is the leading cause of human visual impairment world-wide [1]. Most cases of cataracts are age-related, and likely have both genetic and environmental causes [188]. In addition, mutations in multiple genes are known to underlie congenital cataracts (which arise in children during the first year of life) [189]. However, there are many cases of cataract and other lens disorders for which no causative gene is yet known [190].

Development and adult morphology of the lens is similar in humans and in zebrafish, but there are important differences as well. During the early stages of lens development in humans and most other vertebrates, cells of the surface ectoderm invaginate to form the lens vesicle [191, 192]. In zebrafish lens development, surface ectoderm cells within the lens placode delaminate as a solid cluster of cells rather than a vesicle (Figure 3) [57, 59, 67]. Despite this difference, cell fate analysis demonstrates that the relationship between zebrafish lens placode cell position and cell fate within the mature lens is consistent with the mammalian model [58], which indicates that a common genetic program may exist for lens development in vertebrates.

In zebrafish and humans, the mature lens is made up of tightly-packed lens fibers which are surrounded at the anterior periphery by a proliferative monolayer of lens epithelial cells (Figure 4C). Throughout the life of the organism, epithelial cells near the transition zone exit the cell cycle, migrate, elongate, and degrade their light-scattering organelles to become new secondary fibers [57, 59, 60, 193–195]. In zebrafish, this mature lens state is reached by 72 hpf [58], by which time the lens appropriately focuses an image onto the plane of the retinal photoreceptors [67]. As lens opacity results from many defective cellular processes [189], the early maturity of the zebrafish lens provides a convenient readout for gene requirement by the lens in mutants and knock-down experiments.

There are subtle differences in the mature structure of the lens between zebrafish and humans. The epithelial cell layer on the surface of the mature zebrafish lens extends further toward the posterior suture; in mammals, epithelial cells end near the lens equator [59]. The shape of the mature zebrafish lens is also more spherical than the human lens, and it is responsible for nearly all refraction to focus light onto the retina, unlike terrestrial vertebrates in which much of the light refraction is performed by the cornea [56, 196, 197].

When genes whose mutation causes human congenital cataracts are knocked down in zebrafish embryos, cataracts or other lens abnormalities often result (Table 1). Zebrafish therefore provide a useful model to characterize the molecular mechanism of cataract formation. Human mutations in transcription factors *PITX3* and *FOXE3* cause various anterior segment disorders, including cataracts [198, 199]. Expression of both of these genes has been knocked down in zebrafish via antisense morpholino [200–203], and for both genes this results in lens dysmorphogenesis. Further experiments utilizing these morpholino-injected embryos demonstrated that that *foxe3* is genetically downstream of *pitx3* in the zebrafish lens [202].

Zebrafish with lens defects identified in large-scale mutagenesis screens can also provide valuable information. Proteins known as crystallins contribute to the transparency and refractive power of the lens and cornea [187], and mutations in many lens crystallins are associated with cataracts in human patients [204]. The zebrafish mutant *cloche* (the genetic basis of which is unknown) presents with cataracts as well as severe vascular defects [205,

206]. In these mutants, both *αA-crystallin* mRNA and protein expression is decreased, and the γ-Crystallin present within the lens is predominantly insoluble. Both the cataract phenotype and γ-Crystallin insolubility were rescued in *cloche* mutants by overexpression of *αA-crystallin* by mRNA injection, likely due to the protein chaperone activity of αA-crystallin [205]. This study demonstrated the requirement for αA-crystallin for proper lens development, as well as providing an exciting example of cataract prevention.

The lens capsule is a transparent basement membrane which completely encloses the lens. Laminin is a component of the lens capsule, and the expression of specific Laminin subunits is conserved between zebrafish and humans [207]. One component of zebrafish and human lens capsules is Laminin-111, a heterotrimeric protein made up of Lama1, Lamb1, and Lamc1 subunits [105, 208–212]. None of these subunit genes have so far been associated with an ocular disease in humans, although humans with mutations in *LAMB2* have lens defects including abnormal shape and cataract [213, 214]. However, zebrafish mutants or morphants in *lama1, lamb1*, and *lamc1* all present with defective lens phenotypes [104, 105, 135, 209, 210, 215–219].

In *lama1* morpholino-injected embryos, the lens degenerates completely by 48 hpf [210], and in various homozygous alleles of *lama1* mutants, the lens is dysmorphic and eventually degenerates completely [209, 219]. Homozygous mutants in *lamb1* or *lamc1* present with very similar lens phenotypes characterized by lens dysplasia at 5 dpf [105], and the lens degenerates completely by 6–9 dpf [105, 219]. Zebrafish deficient in subunits of Laminin-111 provide a useful model system to elucidate the roles for laminin-containing extracellular matrix in the lens. For instance, one study found that reduced Focal Adhesion Kinase signaling likely underlies the lens phenotype in *lama1* mutants [209].

B. Cornea

As the most anterior structure within the eye, the transparent cornea provides protection and, in terrestrial vertebrates, refraction of incoming light [56, 196, 197]. Major causes of corneal blindness include the infectious disease Trachoma, vitamin A deficiency, and injury [1]. Human genetic corneal dystrophies, though less common, are another cause of blindness due to corneal opacity [220].

In humans and in zebrafish, the mature cornea contains five major layers: the stratified corneal epithelium at the anterior, Bowman's Layer, the stroma, Descemet's membrane, and the corneal endothelium [60, 62, 63, 66, 221]. At 72 hpf in zebrafish, a rudimentary cornea consisting of a two-cell-layer epithelium, an acellular stroma, and the endothelium is in place [60, 62]. Bowman's layer is formed by 5 dpf, and the zebrafish cornea (which is much thinner than the human cornea) is fully mature at 2 months post fertilization [62].

Many shared proteins are expressed in both the human and zebrafish cornea [62], although many of the highly-expressed corneal crystallins are taxon-specific [222]. In most mammals, including humans, the most abundant corneal crystallin is Aldehyde Dehydrogenase 3a1 [223]. In contrast, 50% of soluble protein in zebrafish adult cornea is made up of Scinderin-Like Protein A (also known as C/L-Gelsolin and Gelsolin-Like 1) [224].

There are several human corneal dystrophies which lead to opacity of the cornea. For many, the responsible gene is known, but few knockout or transgenic mouse mutants in the particular defective gene have been reported [220]. Some of these genes, including *pip5k3* (Fleck Corneal Dystrophy, [225, 226]) and *keratocan* (Cornea Plana, [227, 228]) are expressed in the zebrafish cornea. Using blastula-stage transplants (see introduction), it is possible to study the function of genes whose mutation would otherwise be embryonic lethal in the mature zebrafish cornea [209]. Semina and colleagues [209] utilized this technique to

show that loss of functional *lama1* in eye tissues leads to focal corneal dysplasia in adult zebrafish. Utilizing early-stage lens ablation, the authors further showed that corneal and other eye defects in *lama1* mutants are not a secondary consequence of lens degeneration.

C. Glaucoma

Second in prevalence only to cataracts in cases of world-wide human blindness [1], glaucoma encompasses a group of related progressive diseases characterized by death of retinal ganglion cells and subsequent degeneration of the optic nerve which leads to irreversible blindness [229]. Although many genes are associated with human cases of glaucoma, incomplete penetrance within families indicates that the various causes of glaucoma are multigenic [230].

Elevated intraocular pressure (IOP) is a major risk factor associated with glaucoma [231, 232]. IOP results from the balance between aqueous humor secretion (from the ciliary epithelium) and outflow, with defective outflow being the most common cause of elevated IOP [233, 234]. In humans, aqueous humor provides nutrients and removes waste products, and its exit from the anterior segment is through the trabecular meshwork outflow pathways [235].

Although one group has advised caution in using zebrafish as a model for glaucoma research due to the ultrastructural dissimilarity between the mammalian trabecular meshwork and the zebrafish annular ligament [65], a second group has more recently utilized aqueous humor tracing experiments to meticulously detail the outflow pathway in the zebrafish eye [61]. Gray and colleagues found that the appropriate zebrafish analog to the mammalian outflow pathway is in fact the ventral canalicular network, and not the annular ligament [61]. Further, the authors found that the ultrastructure of this region was overall quite similar in zebrafish and humans, although the aqueous humor flow pathway in zebrafish is vectorial (flowing from dorsal to ventral) rather than circumferential as in humans. Despite this difference, the overall similarities in aqueous humor outflow tissue structure make the zebrafish a potentially valuable model organism for human glaucomas.

The zebrafish is an ideal model system in which to study multigenic traits [236]. In addition, methodology for measuring zebrafish IOP has been developed, and average intraocular pressure ranges are similar in mammals and zebrafish [64]. Utilizing this technique, the zebrafish mutant *brass* (genetic basis unknown) was shown to have elevated IOP along with anterior segment defects [64]. Although *brass* zebrafish do not develop glaucoma, their genetic background could be used to uncover glaucoma modifier loci [64].

In humans, mutation of the gene *FOXC1* is associated with Axenfeld-Reiger Syndrome, which includes glaucoma in some individuals [237–239]. Zebrafish *foxc1* is expressed in the anterior segment and periocular mesenchyme [236]. By utilizing morpholinos to knock down *foxc1* expression in zebrafish embryos along with other techniques, one study has provided evidence that FoxC1 directly activates expression of *fgf19* within the periocular mesenchyme [240]. Fgf19 goes on to activate Fgfr4 receptors, and this interaction is required for appropriate anterior segment development [240]. Reduced *foxc1* expression during zebrafish eye development also results in reduced expression of the cellular homeostasis and apoptosis regulator *foxo1*, and in increased apoptosis within the zebrafish eye [241].

Mutation of the gene *lmx1b* in humans is associated with Nail-Patella syndrome and an increased susceptibility to glaucoma [242]. Similar to the expression pattern in mice [243], zebrafish orthologues of this gene are expressed in cells of the periocular mesenchyme [103]. Reduction of *lmx1b* expression in zebrafish with morpholinos resulted in early eye

morphological defects, enhanced ocular FGF activity, and in altered expression of two genes also implicated in glaucoma: *foxc1* and *pitx2* [103, 239, 244, 245]. Results from the study further suggest that *lmx1b*-expressing cells in the zebrafish eye have migration defects in the absence of *lmx1b* expression [103].

IV. Concluding Remarks

The zebrafish has emerged as a powerful model system for the study of the cellular and molecular underpinnings of human ocular pathologies. Large-scale genetic screens have identified genes whose study is relevant for understanding both eye development and disease, while the continual development of tools for the manipulation of gene function *in vivo* has enabled researchers to study their functions during eye development and maintenance. Current and future research efforts utilizing the zebrafish system promise to continue to provide important insights into human ocular conditions and contribute to the discovery and development of relevant therapeutics.

Acknowledgments

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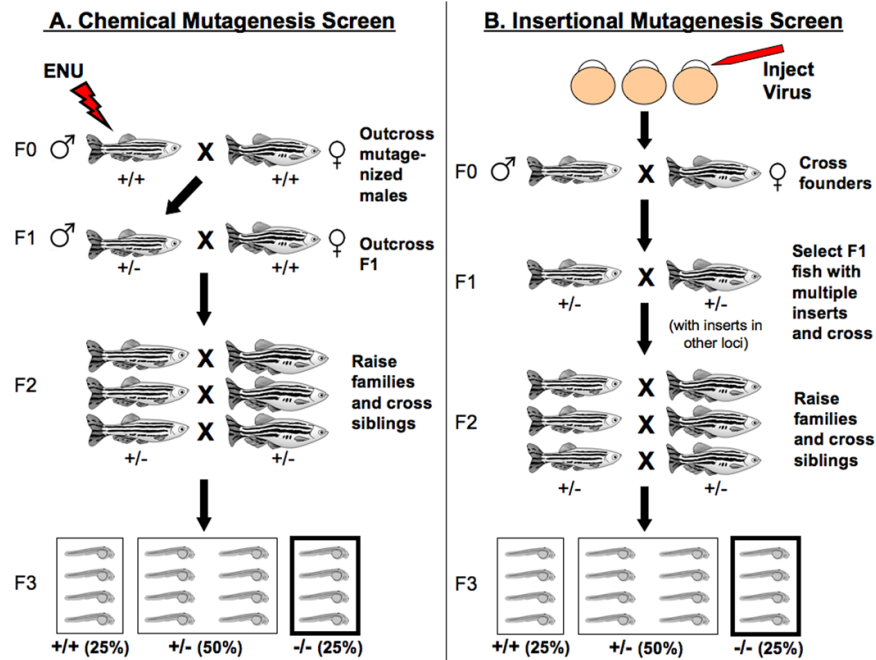
Figure 1. Chemical versus insertional mutagenesis screens in zebrafish

In chemical screens (A), the mutagen ethynitrosourea (ENU) is used to induce mutations in the adult male germline. The mutagenized males (F0 generation) are then out-crossed to generate a heterozygous F1 population. F1 progeny are crossed again to wild-type fish, giving rise to F2 families that carry a specific mutation. F2 siblings are bred to each other in order to generate an F3 generation out of which 25% will be homozygous for the mutation [284, 285]. In an insertional screen (B), a retrovirus is injected into fertilized eggs to generate transgenic adults (F0) that will be subsequently bred to generate an F1 generation with multiple viral inserts. PCR and Southern blot analysis is then performed in order to identify F1 fish that carry multiple inserts. As in a chemical screen, F1 fish are bred to generate F2 families; those will be subsequently bred to siblings to give rise to F3 progeny that will be screened for physiological and/or behavioral phenotypes [40, 285].
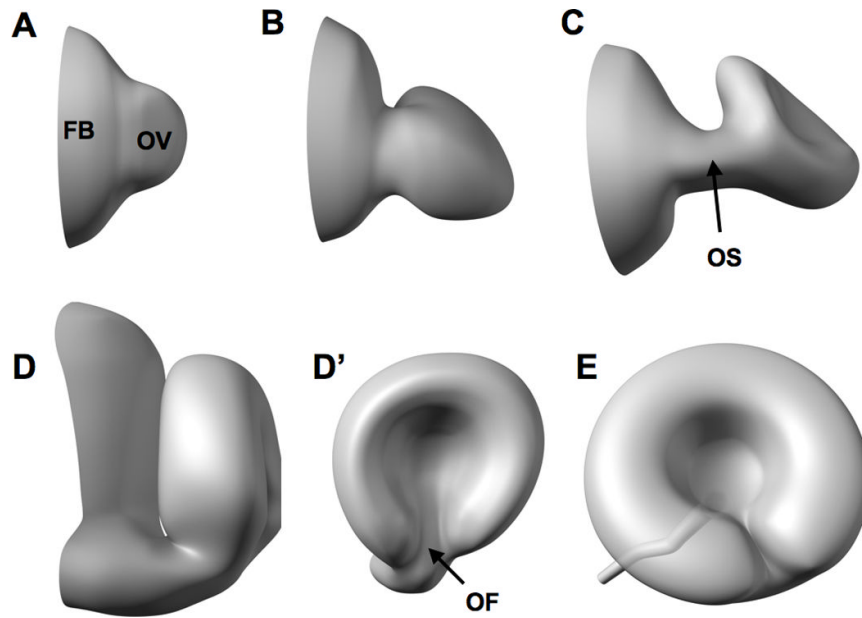
Figure 2. Development and morphogenesis of the zebrafish eye

Eye development commences around 12 hpf as the optic vesicle (OV) evaginates from the forebrain (FB) (A). The optic vesicle then elongates into a flattened wing-like structure at around 16 hpf (B) that is attached to the forebrain through a transient structure called the optic stalk (OS in C). The eye subsequently rotates and invaginates (C) to form the 'optic cup' at around 24 hpf as depicted in D (anterior view) and D' (lateral view). Morphogenesis of the embryonic eye is mostly complete by 48 hpf as the optic fissure (OF in D') is closed and neurogenesis of the retina is underway [54, 68, 286].
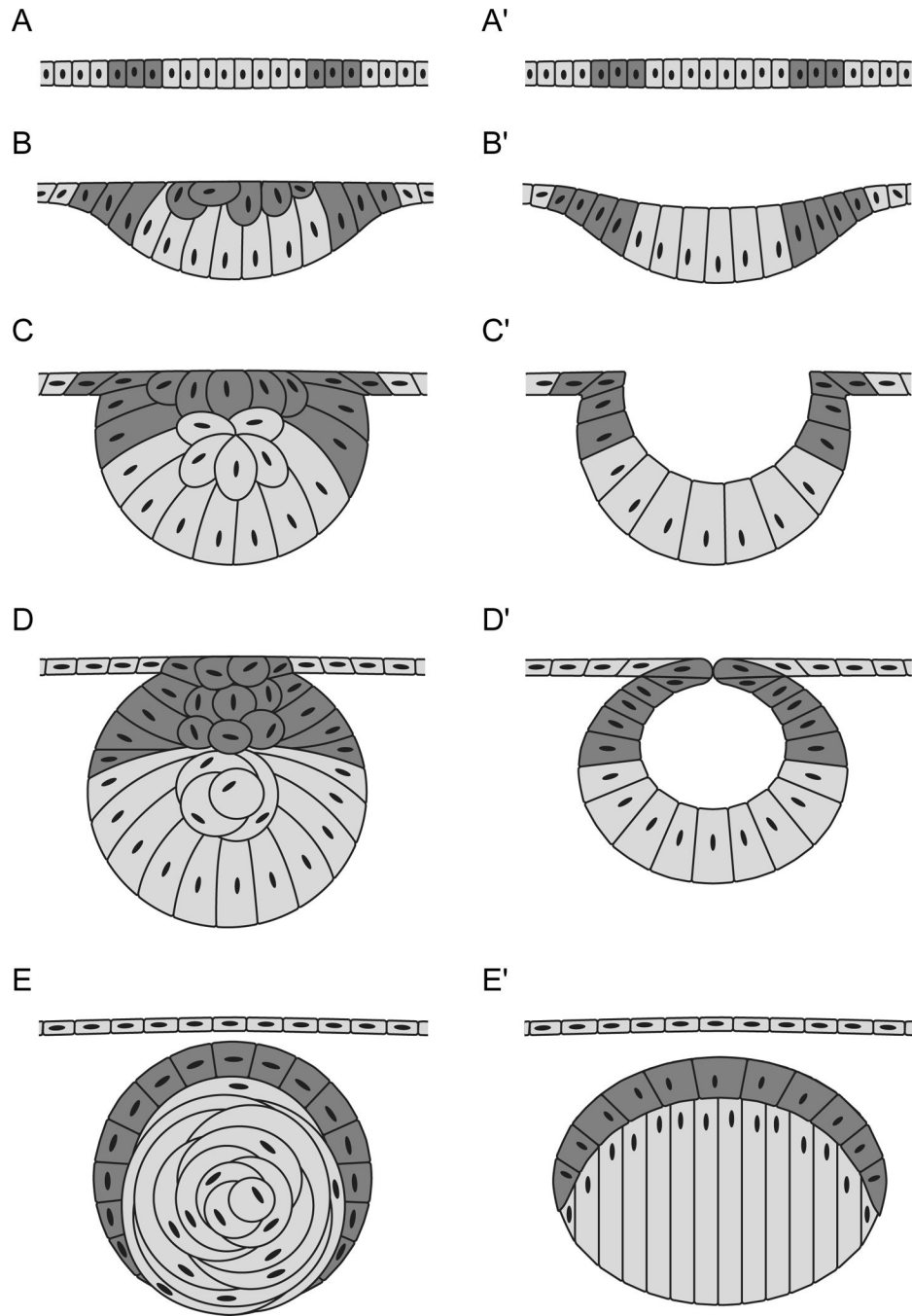
A

A'

B

B'

C

C'

D

D'

E

E'

Figure 3. Early lens development in zebrafish and mouse
After [58]. In zebrafish (A–E) and mouse (A'–E'), the surface ectoderm overlying the optic cup (A, A') thickens to form the lens placode (B, B'). In zebrafish, the lens mass delaminates from the surface ectoderm (C, D), while in mouse the lens placode evaginates to form the lens vesicle (C', D'). Primary lens fibers in the zebrafish elongate within the lens mass in a circular fashion (E), whereas the primary lens fibers in the mouse elongate to the anterior to fill the lens vesicle space (E'). The remaining surface ectoderm becomes the corneal epithelium in both zebrafish and mouse. [57, 59, 67, 191]. Anterior is to the left.
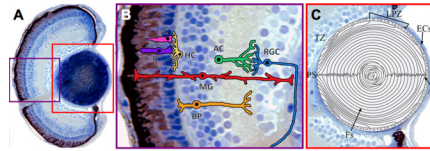
Figure 4. Structure of the zebrafish eye
(A) Transverse histological section of a wild-type zebrafish eye at 5 dpf. (B) Illustration of the major neuronal and glial cell types in the zebrafish retina. Rods and cones relay sensory input to retinal interneurons (Horizontal and Bipolar Cells). Following synaptic interactions with Amacrine Cells, the information is passed to the output neurons, the Ganglion Cells. Müller glia perform multiple functions in the retina, including maintaining retinal health and structure (see 'Müller Glia' section in text). C, Cones. R, Rods. HC, Horizontal Cells. BP, Bipolar. AC, Amacrine Cells. RGC, Ganglion Cells. MG, Müller Glia. (C) Diagram of the zebrafish lens at 5 dpf. Lens epithelial cells surround the anterior periphery; proliferation of these cells is mainly restricted to the lateral proliferative zone. Epithelial cells at the transition zone exit the cell cycle, migrate, elongate, and degrade their light-scattering organelles to become new secondary fibers. Tightly-packed lens fibers which extend from the posterior suture to the anterior suture make up the bulk of the lens. [57–60, 193–195]. ECs, lens epithelial cells. LPZ, lateral proliferative zone. TZ, transition zone. Fs, lens fibers. AS, anterior suture. PS, posterior suture.

Figure 5. Structure of rod and cone photoreceptors
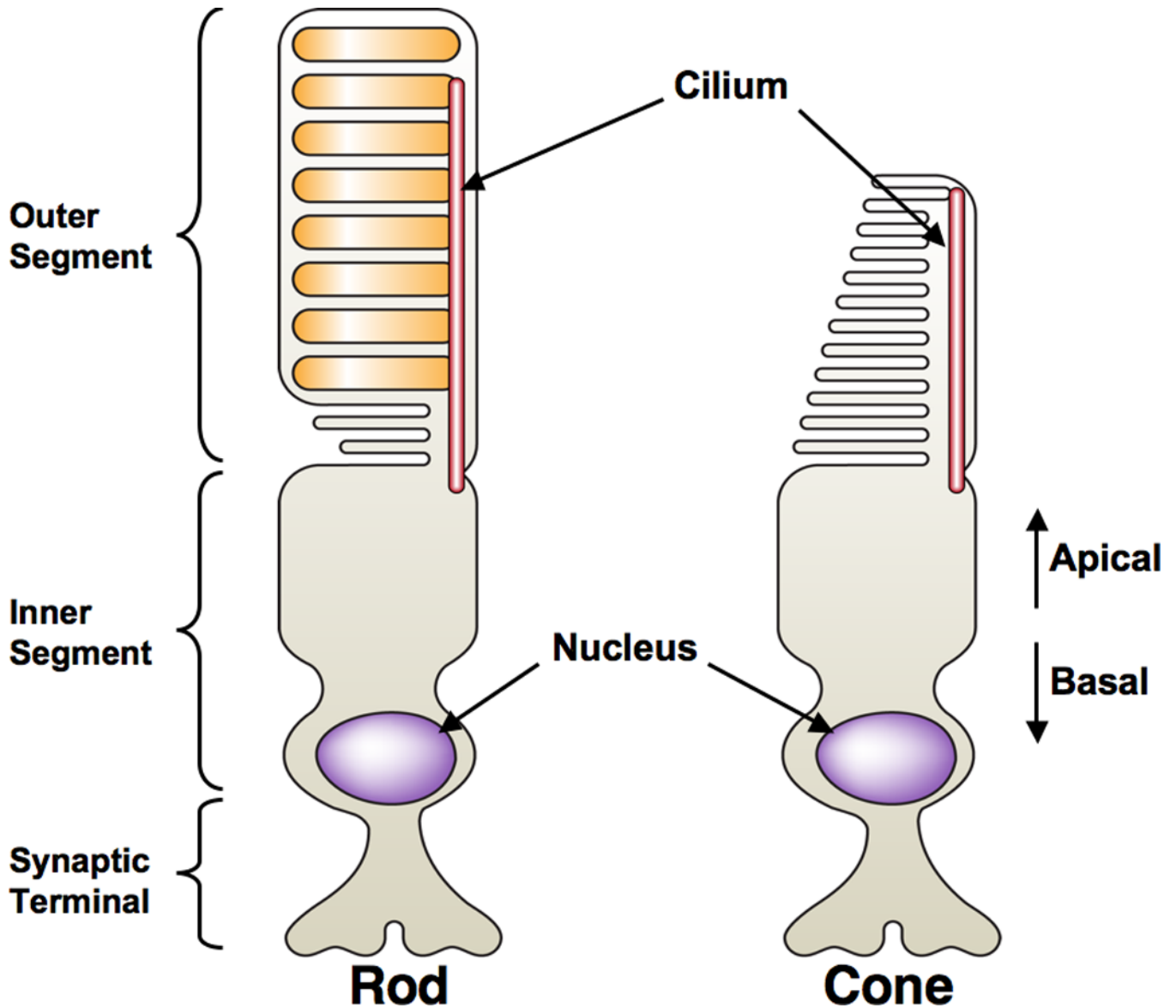
Photoreceptor cells are composed of an outer segment that contains visual pigment-bound membrane disks. The inner segment is the polarized cell body of the photoreceptor, where the nucleus resides basally and the cilium originates apically and extends into the OS. The synaptic terminal forms synaptic connections to the interneurons of the retina, relaying visual input [71, 287].

Table 1

Zebrafish mutants and morphants with ocular phenotypes relevant to human disorders and associated pathologies. Due to space limitations, not all relevant mutant and morphant zebrafish are included in this table.

Gene	Mutant / Morpholino	Ocular Phenotype	References	Associated Human Ocular Disease*	OMIM
Coloboma					
adenomatous polyposis coli (apc)	Mutant	Coloboma; defects in optic vesicle patterning and optic fissure closure	[98]	Familial Adenomatous Polyposis	175100
bcl6 co-repressor (bcor)	Morpholino	Coloboma; microphthalmia	[246]	Oculofaciocardiodental and Lenz microphthalmia	300485
cadherin 2 neuronal (cdh2, glass onion)	Mutant	Coloboma; optic fissure closure defect	[247]	—	—
laminin,β1 (lamb1, lamb1^{hi1113bTg}), laminin,γ1 (lamc1, lamc1^{hi3890Tg})	Mutant	Coloboma; basement membrane defects	[105]	—	—
paired box gene 2a (pax2a, no isthmus)	Mutant	Coloboma	[81]	Renal-coloboma Syndrome	167409
patched1 (ptc1, blowout)	Mutant	Coloboma; defects in optic stalk morphogenesis	[84, 85]	—	—
thioredoxin-related transmembrane prtein 3 (tmx3)	Morpholino	Microphthalmia; coloboma	[248]	Microphthalmia and coloboma	—
transcription factor ap2 alpha (tfap2a)	Morpholino	Coloboma	[249]	Branchio-Oculo-Facial Syndrome	107580
zinc family member 2a (zic2a)	Morpholino	Coloboma; defects in optic stalk morphogenesis and optic vesicle patterning	[88]	—	—
zinc finger proteins 703 and 503 (znf703,503, also known as nlz1 and nlz2)	Morpholino	Coloboma; defects in optic vesicle patterning and optic fissure closure	[79]	—	—
Photoreceptors					
cone transducin a (tca, no optokinetic response f)	Mutant	Reduced sensitivity to light by cones	[250]	—	—
crumbs homolog 2 (crb2, oko meduzy)	Mutant	Photoreceptor defects	[119]	—	—
crumbs homolog 2, like (crb2l)	Morpholino	Photoreceptor defects	[119]	—	—
dihydrolipoamide S-acetyltransferase (pdhe2, no optokinetic response a)	Mutant	Blindness; photoreceptor synaptic transmission defects	[133, 136]	Pyruvate Dehydrogenase Deficiency	—
dynactin 1a (dctn1a, mikre oko)	Mutant	Retinal degeneration	[123, 251]	—	—
dynactin 2 (p50), (dctn2, ale oko)	Mutant	Retinal degeneration	[120]	—	—
erythrocyte membrane protein band 4.1-like 5 (epb4l5, mosaic eyes)	Mutant	Photoreceptor defects	[111, 112]	—	—

Gene	Mutant / Morpholino	Ocular Phenotype	References	Associated Human Ocular Disease*	OMIM
fleer (flr)	Mutant	Rod outer-segment defects	[129, 252]	—	—
intraflagellar transport proteins, 57, 80, 88, and 172 (ift57^{hi3417Tg}, oval(ift88), ift172^{hi2211Tg})	Mutants (*ift57, 88, 172*), Morpholino (*ift80*)	Outer segment defects; retinal degeneration	[104, 126, 127, 253]	—	—
membrane protein, palmitoylated 5a (mpp5a, nagie oko)	Mutant	Disrupted RPE; retinal laminaiton defects; photoreceptor defects	[254, 255]	—	—
Novel protein (partial optokinetic response b)	Mutant	Cone degeneration	[256, 257]	—	—
phosphodiesterase 6 alpha (pde6a, eclipse)	Mutant	Cone degeneration	[258]	—	—
protein kinase C iota (prkci, heart and soul)	Mutant	Photoreceptor morphogenesis defects	[259]	—	—
protocadherin 15b (pcdh15b)	Morpholino	Photoreceptor defects; visual function defects	[260]	Usher Syndrome	605514
TNF receptor-associated factor 3 interacting protein (traf3ip, elipsa)	Mutant	Photoreceptor defects; visual defects	[128, 129]	—	—
Unknown (*brudas*)	Mutant	Photoreceptor defects	[129]	—	—
Unknown (*niezerka*)	Mutant	Photoreceptor defects	[129, 261]	—	—
Unknown (*nightblindness a,b,e,f,g*)	Mutant	Visual function defects; retinal degeneration (*nba, nbe, nbf*)	[137, 138, 141]	—	—
Other Retinal Phenotypes					
patched2 (ptc2, leprechaun)	Mutant	Müller glial reactivity; vitreo-retinal abnormalities	[165]	Basal Cell Naevus Syndrome (BCNS)	601309
phosphatase and tensin homolog b (ptenb)	Mutant	Ocular tumors	[262]	—	—
RPE					
choroideremia (chm)	Mutant	Retinal degeneration; RPE defects	[145, 146]	Choroideremia	300390
protein kinase C iota (prkci, heart and soul)	Mutant	RPE morphogenesis defects	[259]	—	—
silver homolog a (silva, fading vision)	Mutant	RPE defects; photoreceptor defects	[158]	—	—
vacuolar protein sorting 18p (vps18, vps18p^{hi2499aTg})	Mutant	Melanosome maturation defects; reduced visual function	[149]	—	—
vacuolar protein sorting 39 homolog (vps39, leberknodel)	Mutant	RPE vesicle traffic defects; PR defects	[148]	—	—
v-ATPase complex (multiple genes)	Mutants	Melanosome defects; photoreceptor outer segment defects;	[153]	—	—
Unknown (*bleached*)	Mutant	Pigmentation defect; blindness; retinal degeneration	[155]	—	—

Gene	Mutant / Morpholino	Ocular Phenotype	References	Associated Human Ocular Disease*	OMIM
Unknown (fade out)	Mutant	RPE defects; photoreceptor defects	[156]	—	—
Unknown (gantenbein)	Mutant	Cone dystrophy; RPE degeneration	[154]	—	—
Hyaloid Vasculature					
forkhead box C1a (foxc1a) + forkhead box C1b (foxc1b)	Morpholino (coinjection)	reduced hyaloid basement membrane integrity	[186]	Axenfeld-Reiger Syndrome, glaucoma	601090
heparan sulfate 6-O-sulfotransferase 2 (hs6st2)	Morpholino	aberrant patterning of hyaloid vasculature	[185]	—	—
laminin, α1 (lama1)	Morpholino	hyaloid vasculature dysmorphogenesis	[209]	—	—
laminin, α1 (lama1, bashful)	Mutant	no hyaloid vasculature	[185]	—	—
mab-21-like 2 (mab21l2)	Morpholino	reduced hyaloid vasculature	[185]	—	—
microfibrillar-associated protein 2 (mfap2)	Morpholino	reduced hyaloid vasculature branching	[185]	—	—
plexin D1 (plxnd1, out of bounds)	Mutant	aberrant patterning of hyaloid vasculature	[185]	—	—
syndecan 2 (sdc2)	Morpholino	no vasculature on the lens	[185]	—	—
Unknown (fused eyes)	Mutant	no hyaloid vasculature	[185]	—	—
Unknown (margin affected)	Mutant	reduced, then absent hyaloid vasculature	[185]	—	—
Unknown (platinum)	Mutant	premature detachment of hyaloid vasculature from lens	[185]	—	—
Lens					
cadherin 4, retinal (cdh4)	Morpholino	Small opaque lens	[263]	—	—
CDP-diacylglycerol-inositol 3-phosphatidyltransferase (cdipt, lens opaque)	Mutant	Lens cell hyperproliferation; lens degeneration	[264–266]	—	—
choroideremia (chm)	Mutant	Small opaque lens	[217]	Choroideremia	300390
coatomer protein complex, subunit ζ1 (copz1, copz1hi528Tg)	Mutant	Cortical lens defects	[104]	—	—
connexin 48.5 (cx48.5)	Morpholino	Cataracts and small lens	[267]	Cataracts	121015
decapentaplegic and Vg-related 1 (dvr1)	Morpholino	Retention of nuclei in lens fibers	[268]	—	—
fibroblast growth factor 19 (fgf19)	Morpholino	Defective lens cell survival and differentiation	[240, 269]	—	—
forkhead box E3 (foxe3)	Morpholino	Lens dysmorphogenesis; epithelial cell hyperproliferation; defective fiber differentiation	[202, 203]	Congenital primary aphakia, Peter's anomaly, Cataracts	601094

Gene	Mutant / Morpholino	Ocular Phenotype	References	Associated Human Ocular Disease*	OMIM
growth differentiation factor 6a (gdf6a)	Morpholino	Cortical lens defects; defective lens gene expression	[270, 271]	Microphthalmia	601147
heat shock cognate 70-kd protein (hsp70)	Morpholino	Immature lens	[272]	—	—
heat shock transcription factor 1 (hsf1)	Morpholino	Small lens	[273]	—	—
integrator complex subunit 7 (ints7, ints7hi1548Tg, ints7hi3649Tg)	Mutant	Devere lens disorganization	[104]	—	—
laminin, α1 (lama1)	Morpholino	Lens degeneration	[210]	—	—
laminin, α1 (lama1, bashful)	Mutant	Lens degeneration, focal corneal dysplasia	[209, 215, 216, 219, 264]	—	—
laminin, β1 (lamb1, grumpy, lamb1hi1113bTg)	Mutant	Cortical lens defects; lens dysplasia	[105, 217, 219]	—	—
laminin, γ1 (lamc1, sleepy, lamc1hi3890Tg)	Mutant	Cortical lens defects; lens dysplasia and degeneration	[104, 105, 219]	—	—
lengsin, lens protein with glutamine synthetase domain (lgsn)	Morpholino	Lens dysmorphogenesis; lens fiber defects	[274]	—	—
mab-21-like 2 (mab2112)	Morpholino	Lens cell death	[275]	—	—
paired box gene 2a (pax2a, no isthmus)	Mutant	Small opaque lens by 6 dpf	[217]	Coloboma	167409
paired box gene 6b (pax6b)	Morpholino	Variable phenotype: small lens to absent lens	[276]	Peter's anomaly, Aniridia	607108
paired box gene 6b (pax6b, sunrise)	Mutant	Small lens	[277, 278]	Peter's anomaly, Aniridia	607108
paired-like homeodomain transcription factor 3 (pitx3)	Morpholino	Lens dysmorphogenesis: disordered epithelial cells, defective fiber differentiation, fiber cell death	[200–202]	Cataracts, Anterior segment dysgenesis	602669
patched 1 (ptc1)	Morpholino	Small, dysplastic lens	[84]	—	—
retinal homeobox gene 3 (rx3, chokh)	Mutant	Small lens, no retina	[279]	Anophthalmia/Microphthalmia	601881
RNA binding motif 42 (rbm42, rbm42hi2735ATg)	Mutant	Cortical lens defects	[104]	—	—
syndecan 2 (sdc2)	Morpholino	Small lens	[185]	—	—
ubiquitin-like, containing PHD and RING finger domains, 1 (uhrf1, uhrf1hi272Tg, uhrf1hi3020Tg)	Mutant	Lens disorganization and degeneration	[104]	—	—
WD repeat domain 36 (wdr36, wdr36hi3630aTg)	Mutant	Thickening of lens epithelium; cortical lens defects; lens degeneration	[280]	Primary open-angle glaucoma	609669
Unknown (bumper)	Mutant	Lens epithelial cell hyperproliferation, lens fiber degeneration	[135, 278, 281]	—	—
Unknown (chiorny)	Mutant	Small lens	[282]	—	—
Unknown (cloche)	Mutant	Opaque lens with retained nuclei	[205]	—	—

Gene	Mutant / Morpholino	Ocular Phenotype	References	Associated Human Ocular Disease*	OMIM
Unknown (disrupted lens)	Mutant	Disorganized lens fibers	[215, 264]	—	—
Unknown (dou yan)	Mutant	Small lens	[283]	—	—
Unknown (korinthe)	Mutant	Lens degeneration	[278]	—	—
Unknown (margin affected)	Mutant	Small lens	[185]	—	—
Unknown (platinum)	Mutant	Small lens	[185]	—	—
Unknown (rosine)	Mutant	Lens degeneration	[278]	—	—
Unknown (yol)	Mutant	Lens degeneration	[164]	—	—

*
Online Mendelian Inheritance in Man (OMIM) entries are listed if the disrupted zebrafish gene is orthologous to a human gene associated with an ocular disease.